

SPECIFICATION AMENDMENTS

Replace the paragraph beginning at page 2, line 1 with:

In the present invention, the term "plasminogen activator" should not be limited, and should be considered to mean any compounds which can convert inactivate plasminogen to the protease plasmin. For example, a plasminogen activator includes a tissue-type plasminogen activator (tPA), urokinase (UK), pro-urokinase, streptokinase, acylated streptokinase/tPA conjugates, etc.

Replace the paragraph beginning at page 2, line 7 with:

The term "IL-2 inhibitor" used in the present invention should not be limited, and should be considered to mean any one possessing IL-2 inhibitory activity. The A particular example is the one possessing possesses an inhibitory activity on the production of IL-2. And the other is the one that Another example inhibits the transmission of IL-2 signal.

Replace the paragraph beginning at page 2, line 12 with:

The preferable "effect caused by IL-2 inhibitor" is a neuroprotective neuroprotective activity. Particularly, "the effect caused by IL-2 inhibitor" may be the treatment and prevention of acute or chronic cerebral neurodegenerative diseases, such as cerebral ischemic diseases and/or brain damage caused by ischemia.

Replace the paragraph beginning at page 2, line 17 with:

Preferable A preferable "IL-2 inhibitor" is, for example, the tricyclic macrolide shown in EP-0184162, WO89/05303, WO93/05058, WO96/31514, and so on, the disclosure disclosures of which is are incorporated herein by reference. It is well known that those tricyclic macrolides have strong IL-2 inhibitory activity.

Replace the paragraph beginning at page 5, line 7 with:

Preferable protective groups in the "protected hydroxy groups" and the "protected amino" are a 1- (lower alkylthio) - (lower)alkyl group such as a lower alkylthiomethyl group (e.g., methylthiomethyl, ethylthiomethyl, propylthiomethyl, isopropylthiomethyl, butylthiomethyl, isobutylthiomethyl, hexylthiomethyl, etc.), more preferably C₁-C₄ alkylthiomethyl group, most preferably methylthiomethyl group; trisubstituted silyl group such as a tri(lower)alkylsilyl (e.g., trimethylsilyl, triethylsilyl, tributylsilyl, tert-butyldimethylsilyl, tri-tert-butylsilyl, etc.) or lower alkyl-diarylsilyl (e.g., methyldiphenylsilyl, ethyldiphenylsilyl, propyldiphenylsilyl, tert-butyldiphenyl-

silyl, etc.), more preferably tri(C₁-C₄)alkylsilyl group and C₁-C₄ alkyldiphenylsilyl group, most preferably tert-butyldimethylsilyl group and tert-butyldiphenylsilyl group; and an acyl group such as an aliphatic, aromatic acyl group or an aliphatic acyl group substituted by an aromatic group, which are derived from a carboxylic acid, sulfonic acid or carbamic acid.

Replace the paragraph beginning on page 6, line 18 with:

Examples of the aromatic acyl groups include an aroyl group optionally having one or more suitable substituents such as nitro, e.g., benzoyl, toluoyl, xyloyl, naphthoyl, nitrobenzoyl, dinitrobenzoyl, nitronaphthoyl, etc.; and an arenesulfonyl group optionally having one or more suitable substituents such as halogen, e.g., benzenesulfonyl, toluenesulfonyl, xylenesulfonyl, naphthalenesulfonyl, fluorobenzenesulfonyl, chlorobenzenesulfonyl, bromobenzenesulfonyl, iodobenzenesulfonyl, etc.

Replace the paragraph beginning on page 6, line 27 with:

Examples of the aliphatic acyl groups substituted by an aromatic group include ~~an-a~~ (lower)alkanoyl group optionally having one or more suitable substituents such as lower alkoxy or trihalo(lower)alkyl, e.g., phenylacetyl, phenylpropionyl, phenylbutyryl, 2-trifluoromethyl-2-methoxy-2-phenylacetyl, 2-ethyl-2-trifluoromethyl-2-phenylacetyl, 2-trifluoromethyl-2-propoxy-2-phenylacetyl, etc.

Replace the paragraph beginning on page 7, line 21 with:

R²⁴ is an optionally substituted ring system which may contain one or more heteroatoms, ~~Preferable~~ preferably, R²⁴ may be a cyclo(C₅₋₇)alkyl group optionally having suitable substituents, and the following ones can be exemplified.

Replace the paragraph beginning on page 8, line 28 with:

The ~~tricyclic~~ tricyclic compounds (I) and ~~its~~ their pharmaceutically acceptable ~~salt~~ salts for use in accordance with this invention are well known to have excellent immunosuppressive activity, antimicrobial activity and other pharmacological activities and, as such, ~~be~~ are of value for the treatment or prevention of rejection reactions by transplantation of organs or tissues, graft-vs-host diseases, autoimmune diseases, and infectious diseases [EP-A-0184162, EP-A-0323042, EP-A-423714, EP-A-427680, EP-A-465426, EP-A-480623, EP-A-532088, EP-A-532089, EP-A-569337, EP-A-626385, WO89/05303, WO93/05058, WO96/31514, WO91/13889, WO91/19495, WO93/04680, WO93/5059, etc.], the disclosures of which are incorporated herein by reference.

Replace the paragraph beginning on page 11, line 14 with:

The most preferable tricyclic ~~compounds~~ compound (I) is, in addition to FK506, an ascomycin ~~derivatives~~ derivative such as halogenated-ascomycin (e.g., 33-epi-chloro-33-desoxyascomycin), which is disclosed in EP 427,680, example 66a.

Replace the paragraph beginning on page 11, line 19 with:

As the other preferable example of the IL-2 inhibitor, rapamycin [THE MERCK INDEX (12th edition), No. 8288] and its derivatives can be exemplified. Preferred A preferred example of the derivatives is an O-substituted derivative in which the hydroxy in position 40 of formula A illustrated at page 1 of WO 95/16691, incorporated herein by reference, is replaced by -OR₁ in which R₁ is hydroxyalkyl, hydroalkoxyalkyl, acylaminoalkyl and aminoalkyl; for example 40-O-(2-hydroxy)ethyl-rapamycin, 40-O-(3-hydroxy)propyl-rapamycin, 40-O-[2-(2-hydroxy)ethoxy]ethyl-rapamycin and 40-O-(2-acetaminoethyl)-rapamycin. These O-substituted derivatives may be produced by reacting rapamycin (or dihydro or deoxo-rapamycin) with an organic radical attached to a leaving group (for example RX where R is the organic radical which is desired as the O-substituent, such as an alkyl, allyl, or benzyl moiety, and X is a leaving group such as CCl₃C(NH)O or CF₃SO₃) under suitable reaction conditions. The conditions may be acidic or neutral conditions, for example in the presence of an acid like trifluoromethanesulfonic acid, camphorsulfonic acid, p-toluenesulfonic acid or their respective pyridinium or substituted pyridinium salts when X is CCl₃C(NH)O or in the presence of a base like pyridine, a substituted pyridine, diisopropylethylamine or pentamethylpiperidine when X is CF₃SO₃. The most preferable one is 40-O-(2-hydroxy) ethyl rapamycin, which is disclosed in WO94/09010, the disclosure of which is incorporated herein by reference.

Replace the paragraph beginning on page 12, line 17 with:

The tricyclic compounds (I), and rapamycin and its derivatives, may be in a form of ~~its~~ a salt, which includes conventional non-toxic and pharmaceutically acceptable salt such as the salt with inorganic or organic bases, specifically, an alkali metal salt such as sodium salt and potassium salt, an alkali earth metal salt such as calcium salt and magnesium salt, an ammonium salt and an amine salt such as triethylamine salt and N-benzyl-N-methylamine salt.

Replace the paragraph beginning on page 13, line 6 with:

~~Further~~ A further example of the IL-2 inhibitor is cyclosporin and its derivatives such as cyclosporin A, B, C, D, E, F, G, etc, which are shown in THE MERCK INDEX (12th edition), No. 2821, USP 4,117,118, 4,215,199, 4,288,431, 4,388,307, Helv. Chim. Acta. 60, 1568(1977) and 65, 1655(1982), Transplant. Proc. 17, 1362(1985), and so on. Among which, the most preferable one is cyclosporin A. The disclosures of the above references are incorporated herein.

Replace the paragraph beginning on page 13, line 22 with:

For therapeutic administration, plasminogen activators in the present invention is used in the form of a conventional pharmaceutical preparation in admixture with a conventional pharmaceutically acceptable carrier such as an organic or inorganic solid or liquid excipient which is suitable for oral, parenteral or external administration. The pharmaceutical preparation may be compounded in a solid form such as granule, capsule, tablet, dragee, suppository or ointment, or in a liquid form such as solution, suspension or emulsion for injection, intravenous drip, ingestion, eye drop, etc. If needed, there may be included in the above preparation auxiliary substance such as a stabilizing agent, wetting or emulsifying agent, buffer or any other commonly used additives.

Replace the paragraph beginning on page 14, line 5 with:

The plasminogen activators as the effective ingredient may usually be administered in an amount which can activate plasminogen to ~~plasmine~~ plasmin. In particular, it may be a unit dose of 0.001 mg/kg to 500 mg/kg, preferably 0.01 mg/kg to 10 mg/kg, 1 to 4 times a day. However, the above dosage may be increased or decreased according to age, body weight and conditions of the patient or administering method.

Replace the paragraph beginning on page 14, line 18 with:

If advisable, the plasminogen activators can be mixed with the IL-2 inhibitor prior to its use. So, the composition comprising the said plasminogen activators of the present invention may further comprise the IL-2 inhibitor. ~~And optionally~~ Optionally, it comprises further additional ingredients, such as, mycophenolate mofetil (CellCept), steroids, Azathiopurine, and so on.

Replace the title on page 15, line 13 with:

~~Method~~) Method

Replace the paragraph beginning on page 15, line 15 with:

Thrombotic occlusion of the MCA was induced by photochemical reaction as described by Umemura et al., (1993). Briefly, male Sprague-Dawley rats (SLC, Inc.) ~~weighing~~ weighing about 300g were anesthetized with halothane (4% for induction, 1.5% for maintenance). The animals were placed in the lateral position, and the left MCA was exposed by a microsurgical approach. A stable thrombotic occlusion of MCA was produced by a photochemical reaction between intravenously administered photoreactive dye, rose bengal (10 mg/kg) and transmural green light (540nm), which causes endothelial injury followed by platelet activation. The body temperature of animals was maintained at 37.0~38.0 °C using a heating-pad. Twenty-four hours after the ischemic insult, the brain was removed for histopathological assessment with triphenyltetrazolium chloride (TTC) staining. The infarct area was calculated by a computerized image analysis system.

Replace the title on page 16, line 13 with:

~~Results~~ Results

Replace the paragraph beginning on page 17, line 4 with:

So, the present invention provides a useful neuroprotective agent for preventing or treating acute or chronic cerebral neurodegenerative diseases, such as cerebral ischemic diseases and/or brain damage caused by ischemia. So, it is useful when the following diseases or injury occur, ~~that is,~~ cerebral infarction, head injury, hemorrhage in brain such as subarachnoid hemorrhage or intracerebral hemorrhage, cerebral thrombosis, cerebral embolism, cardiac arrest, stroke (such as acute stroke), transient ischemic attacks (TIA), hypertensive encephalopathy, Alzheimer's disease, Huntington's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS) and so on.

Replace the paragraph beginning on page 17, line 16 with:

~~From the another aspect, the~~ The present invention also provides the following inventions:

Replace the paragraph beginning on page 17, line 18 with:

i) A use of an IL-2 inhibitor for manufacturing a medicament for increasing or decreasing an effect caused by plasminogen activator, in which the effect caused by plasminogen activator is a neuroprotective activity or a brain damage appeared in case that plasminogen activator is administered after its proper therapeutic time.

Replace the paragraph beginning on page 17, line 24 with:

ii) A use of ~~a~~ an IL-2 inhibitor and a plasminogen activator for manufacturing a medicament for simultaneous, separate or sequential use for neuroprotective activity.

Replace the paragraph beginning on page 17, line 27 with:

iii) A method for increasing an effect caused by a plasminogen activator, by administering an effective amount of an IL-2 inhibitor to a human being or an animal.

Replace the paragraph beginning on page 18, line 1 with:

iv) A method for preventing and treating acute or chronic cerebral neurodegenerative diseases, by administering an effective amount of a plasminogen activator and an effective amount of an IL-2 inhibitor to a human being or an animal.

Replace the paragraph beginning on page 18, line 5 with:

v) A composition comprising a plasminogen activator and an IL-2 inhibitor as a combined preparation for simultaneous, separate or sequential use for neuroprotective activity.

Replace the paragraph beginning on page 18, line 8 with:

vi) An article of manufacture, comprising packaging material and an IL-2 inhibitor contained within said packaging material, wherein said IL-2 inhibitor is therapeutically effective for increasing or decreasing an effect caused by plasminogen activator, and wherein said packaging material comprises a label or a written material which indicates that said IL-2 inhibitor can be used for increasing or decreasing an effect caused by plasminogen activator.

Replace the paragraph beginning on page 18, line 16 with:

vii) An article of manufacture, comprising packaging material and a plasminogen activator contained within said packaging material, wherein said plasminogen activator is therapeutically effective for increasing an effect caused by an IL-2 inhibitor, and wherein said packaging material comprises a label or a written material which indicates that said plasminogen activator can be used for increasing an effect caused by IL-2 inhibitor.